

(12) INTERNATIONAL APPLICATION PUBLISHED UNDER THE PATENT COOPERATION TREATY (PCT)

(19) World Intellectual Property
Organization
International Bureau



(43) International Publication Date
1 April 2004 (01.04.2004)

PCT

(10) International Publication Number
WO 2004/026327 A1

- (51) International Patent Classification⁷: **A61K 38/00**
- (21) International Application Number:
PCT/US2003/029379
- (22) International Filing Date:
18 September 2003 (18.09.2003)
- (25) Filing Language: English
- (26) Publication Language: English
- (30) Priority Data:
60/412,330 19 September 2002 (19.09.2002) US
- (71) Applicant (*for all designated States except US*): **CORNELL RESEARCH FOUNDATION, INC.** [US/US]; 20 Thornwood Drive, Ithaca, NY 14850 (US).
- (72) Inventors; and
- (75) Inventors/Applicants (*for US only*): **EDELBERG, Jay, M.** [US/US]; 430 East 63rd Street, Apartment 8A, New York, NY 10021 (US). **CAI, Dong, Qing** [CN/CN]; Room 106, No. 36, Su Zhou Yuan, Ji Nan University, Guangzhou 510632 (CN). **HEMPSTEAD, Barbara, L.** [US/US]; 525 East 86th Street, Apartment 9c, New York, NY 10028 (US).
- (74) Agents: **FEIT, Irving, N. et al.; Hoffmann & Baron, LLP**, 6900 Jericho Turnpike, Syosset, NY 11791 (US).
- (81) Designated States (*national*): AE, AG, AL, AM, AT, AU, AZ, BA, BB, BG, BR, BY, BZ, CA, CH, CN, CO, CR, CU, CZ, DE, DK, DM, DZ, EC, EE, EG, ES, FI, GB, GD, GE, GH, GM, HR, HU, ID, IL, IN, IS, JP, KE, KG, KP, KR, KZ, LC, LK, LR, LS, LT, LU, LV, MA, MD, MG, MK, MN, MW, MX, MZ, NI, NO, NZ, OM, PG, PH, PL, PT, RO, RU, SC, SD, SE, SG, SK, SL, SY, TJ, TM, TN, TR, TT, TZ, UA, UG, US, UZ, VC, VN, YU, ZA, ZM, ZW.
- (84) Designated States (*regional*): ARIPO patent (GH, GM, KE, LS, MW, MZ, SD, SL, SZ, TZ, UG, ZM, ZW), Eurasian patent (AM, AZ, BY, KG, KZ, MD, RU, TJ, TM), European patent (AT, BE, BG, CH, CY, CZ, DE, DK, EE, ES, FI, FR, GB, GR, HU, IE, IT, LU, MC, NL, PT, RO, SE, SI, SK, TR), OAPI patent (BF, BJ, CF, CG, CI, CM, GA, GN, GQ, GW, ML, MR, NE, SN, TD, TG).
- Published:**
- *with international search report*
 - *before the expiration of the time limit for amending the claims and to be republished in the event of receipt of amendments*
- For two-letter codes and other abbreviations, refer to the "Guidance Notes on Codes and Abbreviations" appearing at the beginning of each regular issue of the PCT Gazette.*

(54) Title: HOMING PEPTIDES TO RECEPTORS OF HEART VASCULATURE

(57) Abstract: The present invention relates to peptides which selectively or preferentially home to areas of a heart. The invention further relates to conjugates of the homing peptides and uses thereof.

WO 2004/026327 A1

Docket 955-39 PCT

HOMING PEPTIDES TO RECEPTORS OF HEART VASCULATURE

This application asserts priority to U.S. Provisional Application Serial No. 60/415,330 filed on September 19, 2002. The specification of U.S. Provisional Application Serial No. 60/415,330 is hereby incorporated by reference in its entirety.

The invention described in this application was made with funds from the National Institutes of Health, Grant Number R01 AG20918-01. The United States government has certain rights in the application.

BACKGROUND OF THE INVENTION

The vasculature of a heart is comprised of blood vessels, such as arteries and veins, and the microvasculature. The microvasculature includes capillaries, arterioles, and venules, and are comprised of microvasculature endothelial cells. Numerous receptors are present on the cells that constitute the vasculature of a heart. The receptors known to exist on the cells include tumor necrosis factor (TNF) receptors and brain derived neurotrophic factor (BDNF) receptors.

Disease of the heart vasculature, i.e., cardiovascular disease, is the leading cause of morbidity and mortality in older individuals. As the populations ages, the need for optimal geriatric therapies is an increasingly important public health issue. Currently, myocardial infarction in older individuals has a significantly worse prognosis with higher mortality and complication rates than younger individuals. Furthermore, depending, in part, on the extent of vascular damage, an individual can have areas of the heart which are "old" (i.e., relatively unhealthy) and other areas which are "young" (i.e., relatively healthy). Therefore, senescent changes in the cardiovascular system plays an important role in predisposing older areas of the heart or older hearts, to increased vascular pathology.

Therefore, there is a need to determine the condition of a heart and to provide treatments targeted to specific areas of a heart.

SUMMARY OF THE INVENTION

In one aspect, the invention relates to an isolated peptide comprising or consisting of the amino acid sequence QA(Q/E)GQLV (SEQ. ID. NO: 1). The peptide selectively homes to TNF receptor(s) in the vasculature of a heart.

In another embodiment, the invention relates to an isolated peptide comprising or consisting of the amino acid sequence ARRQAV (SEQ. ID. NO: 4). The peptide preferentially homes to BDNF receptor(s) in the vasculature of a heart.

In another embodiment, the invention relates to an isolated peptide comprising or consisting of the amino acid sequence G(R/W)RFIRV (SEQ. ID. NO: 2). The peptide preferentially homes to BDNF receptor(s) in the vasculature of a heart.

In another embodiment, the invention relates to a conjugate comprising a peptide which comprises or consists of the amino acid sequence QA(Q/E)GQLV (SEQ. ID. NO: 1) or functionally equivalent modifications thereof, and a functional moiety. The peptide selectively homes to TNF receptor(s) in the vasculature of a heart.

In another embodiment, the invention relates to a conjugate comprising a peptide which comprises or consists of the amino acid sequence ARRQAV (SEQ. ID. NO: 4) or functionally equivalent modifications thereof, and a functional moiety. The peptide preferentially homes to BDNF receptor(s) in the vasculature of a heart.

In another embodiment, the invention relates to a conjugate comprising a peptide which comprises or consists of the amino acid sequence G(R/W)RFIRV (SEQ. ID. NO: 2) or functionally equivalent modifications thereof, and a functional moiety. The peptide preferentially homes to BDNF receptor(s) in the vasculature of a heart.

In another embodiment, the invention relates to a method for determining a young heart or young areas of a heart vasculature in a mammal. The method comprises administering to the mammal a peptide comprising or consisting of the amino acid sequence QA(Q/E)GQLV (SEQ. ID. NO: 1) or functionally equivalent

modifications thereof, conjugated to a detectable marker, wherein the peptide selectively homes to TNF receptor(s) in the vasculature of the heart and the marker is detected. A disproportionately high binding of the peptide comprising or consisting of QA(Q/E)GQLV (SEQ. ID. NO: 1) indicates a young heart or young areas of a heart vasculature.

In another embodiment, the invention relates to a method for determining an old heart or old areas of a heart vasculature in a mammal. The method comprises administering to the mammal a peptide comprising or consisting of the amino acid sequence ARRQAV (SEQ. ID. NO: 4) or G(R/W)RFIRV (SEQ. ID. NO: 2) or functionally equivalent modifications thereof, conjugated to a detectable marker, wherein the peptide selectively homes to BDNF receptor(s) in the vasculature of the heart and the marker is detected. A disproportionately high binding of the peptide comprising or consisting of ARRQAV (SEQ. ID. NO: 4) or G(R/W)RFIRV (SEQ. ID. NO: 2) indicates an old heart or old areas of a heart vasculature.

In another embodiment, the invention relates to a method for determining the condition of the vasculature of a heart in a mammal. The method comprises administering to the mammal a first peptide comprising or consisting of the amino acid sequence QA(Q/E)GQLV (SEQ. ID. NO: 1) or functionally equivalent modifications thereof, conjugated to a first detectable marker. The first peptide selectively homes to TNF receptor(s) in the vasculature of the heart. The method further comprises administering to the mammal a second peptide comprising or consisting of the amino acid sequence ARRQAV (SEQ. ID. NO: 4) or G(R/W)RFIRV (SEQ. ID. NO: 2) or functionally equivalent modifications thereof, conjugated to a second detectable marker. The second peptide homes to BDNF receptor(s) in the vasculature of the heart. The first and second marker are detected. A disproportionately high ratio of binding of the first peptide to the second peptide indicates a young heart or young areas of the heart vasculature. Alternatively, a disproportionately low ratio of binding of the first peptide to the second peptide indicates an old heart or old areas of the heart vasculature.

In another embodiment, the invention relates to a method for delivering a functional moiety selectively to a young heart or a young area of the heart in a mammal. The method comprises administering to the mammal a conjugate

comprising a peptide which comprises or consists of the amino acid sequence QA(Q/E)GQLV (SEQ. ID. NO: 1) or functionally equivalent modifications thereof, and a functional moiety.

In another embodiment, the invention relates to a method for delivering a functional moiety preferentially to an old heart or an old area of the heart in a mammal. The method comprises administering to the mammal a conjugate comprising a peptide which comprises the amino acid sequence ARRGQAV (SEQ. ID. NO: 4) or G(R/W)RFIRV (SEQ. ID. NO: 2) or functionally equivalent modifications thereof, and a functional moiety.

In another embodiment, the invention relates to a method for discovering mimics of peptides comprising or consisting of amino acid sequence QA(Q/E)GQLV (SEQ. ID. NO: 1) or functionally equivalent modifications thereof. The method comprises determining a three-dimensional structure of peptides comprising or consisting of the sequence; identifying compounds comprising the structure; and determining the capacity of the compounds for selective homing to TNF receptor(s) in the vasculature of a heart. The compounds which home selectively to TNF receptor(s) in the vasculature of the heart are mimics.

In another embodiment, the invention relates to a method for discovering mimics of peptides comprising or consisting of amino acid sequence ARRGQAV (SEQ. ID. NO: 4) or G(R/W)RFIRV (SEQ. ID. NO: 2) or functionally equivalent modifications thereof. The method comprises determining a three-dimensional structure of peptides comprising or consisting of the sequence; identifying compounds comprising the structure; and determining the capacity of the compound for homing to BDNF receptor(s) in a vasculature of a heart. The compounds which home preferentially to BDNF receptor(s) in the vasculature of the heart are mimics.

BRIEF DESCRIPTION OF THE FIGURES

Figure 1. Amino acid sequence of human TNF α Genbank Accession No. 1TNFA (SEQ. ID. NO: 6).

Figure 2. Amino acid sequence of human BDNF Genbank Accession No. 1BNDA (SEQ. ID. NO: 7).

Figure 3. Identification of aging-associated changes in cardiac microvascular epitope binding. (A) ψ Y12 epitope sequence homology to TNF α . (B) Homologous region is highlighted yellow in the three-dimensional structure of TNF α . (C) Representative phage immunostaining patterns in hearts with isolated helper phage and ψ Y12 clones with minimal labeling of the 18-month old hearts (arrows). (D) ψ Y12 phage binding the cardiac microvasculature of 3-month old hearts (arrow). (E) ψ Y12 phage titers of 3- and 18-month old hearts.

Figure 4. Aging-associated alterations in cardiac microvascular TNF α receptor pathways. (A) Representative immunostaining for TNF receptor 1 and 2 in the subepicardium and subendocardium of 3- and 18-month old murine hearts. (B) TNF receptor 1 and 2 densities in the subendocardium of 3- and 18-month old murine hearts (n=3, each). (C) RT-PCR for PDGF-B induction after TNF α treatment of 4- and 24-month old rat CMECs.

Figure 5. Aging-associated alterations in TNF α cardioprotection. (A) Representative immunostaining for PDGF-B in rat hearts injected with TNF α or PBS vehicle 24 h prior to sacrifice. (B) Quantification of PDGF-B density in the injected hearts (n=3, each group). (C) Representative cardiac histology after coronary occlusion: Mason's trichrome staining for myocardial necrosis (14 day post-coronary occlusion) in 4- and 24- month old rat hearts treated with PBS (4- and 24-month old, n=4 and 10, respectively) and TNF α (4-month old, n=4); and (D) tunnel staining for apoptosis (DAB) with hematoxylin counter stain (2 day post-coronary occlusion) in 24-month old hearts treated with TNF α (n=10; 80% mortality vs. PBS 0%; p<0.05). (E) Quantification of myocardial necrosis in rat hearts 14 day post-coronary occlusion.

Figure 6. Identification of aging-associated changes in BDNF homologous phage cardiac microvascular epitope binding. (A) ψ O40 epitope sequence homology to BDNF. (B) Homologous region is highlighted yellow in the 3-D structure of BDNF. (C) Representative phage immunostaining patterns in hearts with isolated

ψ O40 clones with phage binding the cardiac microvasculature of 18-month-old hearts with minimal binding to 3-month-old hearts ($n = 3$, each). (D) ψ O40 phage titers of 3- and 18-month-old hearts ($n = 3$, each). * $P < 0.05$ 3- vs. 18-month-old hearts.

Figure 7A-D. Aging associated alterations in cardiac microvascular BDNF receptor pathways, immunostaining for the full-length receptor as well as the truncated trkB receptor in sections of 3- and 18-month old rat hearts.

Figure 8. BDNF induction of activated macrophage recruitment in the hearts of old but not young rats. (A) Representative histology and immunostains for ED2 and ED1 in 4- and 24-month-old rat hearts injected with BDNF or PBS 24 hr prior to tissue harvest. (B) Quantification of ED1 and ED2 cellular density in the injected hearts ($n=3$, each group). * $P<0.05$ BDNF vs. PBS.

Figure 9. Aging-associated alterations in BDNF cardiac actions. (A) Representative cardiac histology after coronary occlusion: Masson's trichrome staining for myocardial necrosis (14 d post-coronary occlusion) in 4- and 24-month-old rat hearts treated with PBS and BDNF. (B) Quantification of myocardial necrosis in rat hearts 14 d post-coronary occlusion.

Figure 10. Aging-associated BDNF cardiac aneurysm formation post coronary occlusion. (A) Representative cardiac histology (Masson's trichrome) cardiac aneurysm formed in 3/5 18-month-old rats receiving intramyocardial injection of BDNF 1d prior to coronary occlusion. (B) Inflammatory infiltrate in BDNF injected heart.

DETAILED DESCRIPTION OF THE INVENTION

The invention is based on the surprising discovery that a peptide having the amino acid sequence QAQGQLV (SEQ. ID. NO: 3) binds to receptors in the heart vasculature in a mammal, but does not bind significantly to receptors on other organs. Similarly, a peptide having the amino acid sequence ARRQGAV (SEQ. ID. NO: 4) or GRRFIRV (SEQ. ID. NO: 5) binds to receptors in the vasculature of the heart and brain, but do not bind significantly to receptors on other organs.

Also surprisingly, a peptide having the amino acid sequence QAQGQLV (SEQ. ID. NO: 3) binds with more binding capacity to TNF receptor(s) in the vasculature of hearts which are "young" (i.e., healthy) than to TNF receptor(s) in the vasculature of older hearts (i.e., unhealthy). Another discovery is that young hearts have more TNF receptor 1 present than do older hearts. The inventors also surprisingly discovered that treatment of myocardial infarction with TNF α markedly reduced the extent of myocardial infarction in young hearts, but induced apoptosis of cardiac cells in older hearts.

Furthermore, the inventors also discovered that a peptide having the amino acid sequence ARRQAV (SEQ. ID. NO: 4) or GRRFIRV (SEQ. ID. NO: 5) bind to BDNF receptor(s) in the vasculature of older hearts, usually to trkB receptors, and more commonly to truncated trkB receptors. Another discovery is that older hearts have more truncated trkB receptors than do younger hearts. The inventors also surprisingly discovered that treatment of myocardial infarction with BDNF had no effect on myocardial infarctions in young hearts. In older hearts, BDNF increased the extent of myocardial infarction, induced inflammatory infiltration, and aneurysm formation.

As a result of the surprising discoveries described above, the present invention provides isolated peptides which selectively or preferentially home to TNF receptor(s) or BDNF receptor(s) in the vasculature of a heart. Preferably, the homing peptides of the present invention bind to receptors of the microvasculature of a heart, especially on microvasculature endothelial cells.

In one embodiment, a homing peptide comprises the amino acid sequence QA(Q/E)GQLV (SEQ. ID. NO: 1) or a functionally equivalent modification thereof. The amino acid sequence QA(Q/E)GQLV (SEQ. ID. NO: 3) is homologous to a sequence found at amino acid positions 16-22 of human TNF α (Fig. 1). If the homing peptide comprises the amino acid sequence QAQGQLV (SEQ. ID. NO: 3), the amino acid residues at positions 1-2 and 4-6 are identical to the amino acid residues found at positions 16-17 and 19-21 of human TNF α . If the homing peptide comprises the amino acid sequence QAEGQLV (SEQ. ID. NO: 8), the amino acid residues at positions 1-6 are identical to the amino acid residues found at positions 16-20 of human TNF α .

Homing peptides comprising the amino acid sequence QA(Q/E)GQLV (SEQ. ID. NO: 1) selectively home to TNF receptor(s) in the vasculature of a heart.

The TNF receptor(s) can be any receptor which binds TNF α . Examples of TNF receptors include TNF receptor (TNFR) 1, TNFR2 (i.e., p75), and receptors which are homologous to TNFR1 or TNFR2, such as receptors in the TNFR superfamily. Preferably, the TNF receptor is TNFR1.

In another embodiment, a homing peptide comprises the amino acid sequence ARRQGAV (SEQ. ID. NO: 4) or G(R/W)RFIRV (SEQ. ID. NO: 2), or a functionally equivalent modification thereof.

The amino acid sequence ARRQGAV (SEQ. ID. NO: 4) is homologous to a sequence found at amino acid positions 5-11 of human BDNF (Fig. 2). The amino acid residues at positions 1-5 of amino acid sequence ARRQGAV (SEQ. ID. NO: 4) are identical to the amino acids at positions 5-9 of human BDNF (Fig. 2).

The amino acid sequence G(R/W)RFIRV (SEQ. ID. NO: 2) is homologous to a sequence found at amino acid positions 99-105 of human BDNF. If the homing peptide comprises the amino acid sequence GRRFIRV (SEQ. ID. NO: 5), the amino acid residues at positions 1 and 3-6 are identical to the amino acid residues found at positions 99 and 101-104 of human BDNF (Fig. 2). If the homing peptide comprises GWRFIRV (SEQ. ID. NO: 9), the amino acid residues at positions 1-6 are identical to the amino acid residues found at positions 99-104 of human BDNF.

Homing peptides comprising the amino acid sequence ARRQGAV (SEQ. ID. NO: 4) or G(R/W)RFIRV (SEQ. ID. NO: 2) preferentially home to BDNF receptor(s) in the vasculature of a heart.

The BDNF receptor(s) can be any receptor which binds BDNF. Examples of BDNF receptors include, trkB, p75, and the nerve growth factor family of receptors, such as trkA and trkC. Usually, the BDNF receptor is trkB, and more commonly truncated trkB. "Truncated trkB" as used herein means that the trkB receptor lacks the intracellular tyrosine kinase domain. For example, the trkB receptor can lack the entire intracellular tyrosine kinase domain or can lack a portion of the domain.

Typically, the truncated trkB receptor lacks a portion of the domain sufficient to cause loss of kinase activity.

The term "peptide" as used herein means an amino acid sequence having at least five amino acid residues. No distinction will be made between a peptide, polypeptide, or protein. The peptide of the invention can be linear, cyclic, or branched.

The peptides of the invention include functionally equivalent modifications of SEQ. ID. NOs: 1, 2, 3, 4, 5, 8 and 9. A functionally equivalent modification refers to a molecule having a similar, non-identical sequence and the selective or preferential homing property described herein. A functionally equivalent modification can be, for example, two, preferably one deletions and/or substitutions relative to the reference peptide sequence. Any deletions or substitutions in the amino acid sequence of the reference peptide are permitted provided that the peptide of the invention continues to satisfy the functional criteria described above and below.

Substitutions in the amino acid sequence of the reference peptide are preferably with equivalent amino acids. Groups of amino acids known to be of equivalent character are listed below:

- (a) Ala(A), Ser(S), Thr(T), Pro(P), Gly(G);
- (b) Asn(N), Asp(D), Glu(E), Gln(Q);
- (c) His(H), Arg(R), Lys(K);
- (d) Met(M), Leu(L), Ile(I), Val(V); and
- (e) Phe(F), Tyr(Y), Trp(W).

The deletions can include any one or two amino acid residue deletions. Preferably, the deletions are one or two amino acid residues at either the N- or C-terminal of the reference peptide. Most preferably, the deletions are the amino acid residues that are not homologous to TNF α or BDNF.

Therefore, in this specification, when reference is made to amino acid sequences QA(Q/E)GQLV (SEQ. ID. NO: 1), ARRGGQAV (SEQ. ID. NO: 4), or

G(R/W)RFIRV (SEQ. ID. NO: 2), the functionally equivalent modifications are also included.

Preferred amino acid residues for the functionally equivalent modifications of the peptide comprising the amino acid sequence QA(Q/E)GQLV (SEQ. ID. NO: 1) are a Q at amino acid position 3 or a V at amino acid position 7. In an especially preferred embodiment, Q is present at amino acid position 3 and V is present at amino acid position 7.

Similarly, preferred amino acid residues for the functionally equivalent modifications of the peptide comprising the amino acid sequence ARRQGAV (SEQ. ID. NO: 4) are an A at amino acid position 6 or a V at amino acid position 7. In an especially preferred embodiment, A is present at amino acid position 6 and V is present at amino acid position 7.

Likewise, preferred amino acid residues for the functionally equivalent modifications of the peptide comprising the amino acid sequence G(R/W)RFIRV (SEQ. ID. NO: 2) are a W at amino acid position 2 or a V at amino acid position 7. In an especially preferred embodiment, W is at amino acid position 2 and V is at amino acid position 7.

The homing peptides described above can further comprise additional amino acid residues. The additional amino acid residues can be any number such that the peptide selectively or preferentially homes to the receptor(s) described above in the vasculature of a heart. For instance, the homing peptide can be a relatively short peptide. The approximate minimum number of amino acid residues in the peptide can, for example, be 5, 7, 10, 15, 20, 30, or 40 amino acids in length. There is no particular maximum number of amino acid residues for the homing peptides of the peptide invention. For example, a suitable maximum number of amino acid residues in the peptide can be approximately 200, more preferably approximately 175, and even more preferably approximately 150 amino acid residues.

The additional amino acid residues can be any amino acid residue such that the peptide selectively or preferentially homes to the receptor(s) described above in the vasculature of a heart. For example, if the homing peptide comprises the amino acid sequence QA(Q/E)GQLV (SEQ. ID. NO: 1), the additional amino acid residues

can be any one or more amino acid residues that occur naturally at the N-terminal and/or C-terminal side of amino acid residues at positions 16-22 of human TNF α (Fig. 1).

For instance, naturally occurring amino acid residues which can be found N-terminal to amino acid residues at positions 16-22 of human TNF α include the amino acid residues approximately at positions 10-15, 5-15, and 2-15 of human TNF α . Similarly, naturally occurring amino acid residues which can be found C-terminal to amino acid residues at positions 16-22 of human TNF α include the amino acid residues approximately at positions 23-60, 23-120, and 23-150 of human TNF α .

Amino acid residues approximately at positions 10-15, 5-15, and 2-15 of human TNF α are preferably attached to the N-terminus of QA(Q/E)GQLV (SEQ. ID. NO: 1). The amino acid residues approximately at positions 23-60, 23-120, and 23-150 are preferably attached to the C-terminus of QA(Q/E)GQLV (SEQ. ID. NO: 1). It is not necessary to have additional amino acid residues at either or at both the N- and C-terminus of QA(Q/E)GQLV (SEQ. ID. NO: 1). For example, the additional amino acid residues can be attached just at the N-terminus of QA(Q/E)GQLV (SEQ. ID. NO: 1) or just at the C-terminus, or both. In addition, the homing peptide can have no additional amino acid residues attached. Thus, the homing peptide can consist of the amino acid sequence QAQGQLV (SEQ. ID. NO: 3) or QAEGQLV (SEQ. ID. NO: 8).

If the homing peptide comprises the amino acid sequence ARRQGAV (SEQ. ID. NO: 4), the additional amino acid residues can be any one or more amino acid residues that occur naturally at the N-terminal and/or C-terminal side of amino acid residues at positions 5-11 of human BDNF (Fig. 2). For example, naturally occurring amino acid residues which can be found N-terminal to amino acid residues at positions 5-11 of BDNF include the amino acid residues approximately at positions 1-4 and 3-4 of BDNF. Similarly, naturally occurring amino acid residues which can be found C-terminal to amino acid residues at positions 5-11 of human BDNF include the amino acid residues approximately at positions 12-30, 12-50, 12-70, 12-90, and 12-110 of BDNF.

Amino acid residues approximately at positions 1-4 or 3-4 of human BDNF are preferably attached to the N-terminus of ARRQGAV (SEQ. ID. NO: 4). The amino acid residues approximately at positions 12-30, 12-50, 12-70, 12-90, or 12-110 are preferably attached to the C-terminus of ARRQGAV (SEQ. ID. NO: 4). It is not necessary to have additional amino acid residues at either or at both the N- and C-terminus of ARRQGAV (SEQ. ID. NO: 4). For example, the additional amino acid residues can be attached just at the N-terminus of ARRQGAV (SEQ. ID. NO: 4) or just at the C-terminus, or both. In addition, the homing peptide can have no additional amino acid residues attached. Thus, the homing peptide can consist of the amino acid sequence ARRQGAV (SEQ. ID. NO: 4).

If the homing peptide comprises the amino acid sequence G(R/W)RFIRV (SEQ. ID. NO: 2), the additional amino acid residues can be any one or more amino acid residues that occur naturally at the N-terminal and/or C-terminal side of amino acid residues at positions 99-105 of human BDNF (Fig. 2). For example, naturally occurring amino acid residues which can be found N-terminal to amino acid residues at positions 99-105 of BDNF include the amino acid residues approximately at positions 80-98, 60-98, 40-98, 20-98, and 4-98 of BDNF. Similarly, naturally occurring amino acid residues which can be found C-terminal to amino acid residues at positions 99-105 of human BDNF include the amino acid residues approximately at positions 106-109, 106-112, and 106-115 of BDNF.

Amino acid residues approximately at positions 80-98, 60-98, 40-98, 20-98, or 4-98 of human BDNF are preferably attached to the N-terminus of G(R/W)RFIRV (SEQ. ID. NO: 2). The amino acid residues approximately at positions 106-109, 106-112, or 106-115 are preferably attached to the C-terminus of G(R/W)RFIRV (SEQ. ID. NO: 2). It is not necessary to have additional amino acid residues at either or at both the N- and C-terminus of G(R/W)RFIRV (SEQ. ID. NO: 2). For example, the additional amino acid residues can be attached just at the N-terminus of G(R/W)RFIRV (SEQ. ID. NO: 2) or just at the C-terminus, or both. In addition, the homing peptide can have no additional amino acid residues attached. Thus, the homing peptide can consist of the amino acid sequence GRRFIRV (SEQ. ID. NO: 5) or GWRFIRV (SEQ. ID. NO: 9).

The peptides of the invention, including the functionally equivalent modified peptides, are isolated. An isolated peptide is substantially free from other biological components, as well as from materials that may be used in preparation, isolation, characterization or purification of peptides. Examples of other biological components include cellular components, culture media or components (including conditioned media and components thereof, including proteins and nucleic acid molecules), affinity binding agents, such as immunoconjugates or antibodies and other serum components. Examples of materials that may be used in preparation, isolation, or purification of proteins include separation media or membranes, such as nitrocellulose, chromatographic matrices, and electrophoretic gel media, including for instance, polyacrylamide and detergents, such as sodium dodecyl sulfate (SDS).

Preferably, the isolated peptide is at least about 25% to about 90% pure, i.e., free from other peptides and nucleic acid molecules. More preferably, the isolated material is at least about 50% to about 90% pure. Optimally, the isolated peptide is at least about 75% to about 90% pure.

Most preferably, the peptides of the invention, including the functionally equivalent modified peptides, are purified. As used herein, the term "purified" means essentially pure as demonstrated by single band purity on electrophoresis in SDS-polyacrylamide gels (SDS PAGE). Preferably, the purified material is at least about 90% to about 99.9% pure. More preferably, the purified material is at least about 95% to about 99.9% pure. Optimally, the purified material is at least about 99% to about 99.9% pure.

Peptides comprising QA(Q/E)GQLV (SEQ. ID. NO: 1) selectively home to TNF receptor(s) in the vasculature of a heart. A homing peptide is selective for TNF receptor(s) in the heart vasculature if the peptide binds disproportionately to TNF receptor(s) in the heart vasculature, i.e., binds at least two-fold greater to TNF receptor(s) in the heart vasculature than to TNF receptor(s) in non-heart vasculature, such as for example, TNF receptor(s) in the vasculature of the brain or kidney. Preferably, the homing peptide binds at least five-fold greater, and more preferably at least ten times greater to TNF receptor(s) in the heart vasculature than to TNF receptor(s) in non-heart vasculature.

Selective homing can be demonstrated by determining if binding of a homing peptide to TNF receptor(s) in the heart vasculature is specific in accordance with the above definition. For example, the amount of a particular homing peptide bound to TNF receptor(s) in the heart vasculature can be compared to the amount of homing peptide which binds to TNF receptor(s) in non-heart vasculatures. Selective homing can also be determined by showing that peptides that home to the TNF receptor(s) in the heart vasculature are enriched in one or more subsequent rounds of *in vivo* panning.

Peptides comprising ARRQAV (SEQ. ID. NO: 4) or G(R/W)RFIRV (SEQ. ID. NO: 2) preferentially home to BDNF receptor(s) in the vasculature of a heart. A homing peptide is preferential for BDNF receptor(s) in the heart vasculature if the peptide binds disproportionately to BDNF receptor(s) in the vasculature of the heart or brain, i.e., binds at least two-fold greater to BDNF receptor(s) in the heart or brain vasculature than to BDNF receptor(s) in non-heart or non-brain vasculatures, such as for example, BDNF receptor(s) in the vasculature of the kidney. Preferably, the homing peptide binds at least five-fold greater, and more preferably at least ten times greater to BDNF receptor(s) in the heart or brain vasculature than to BDNF receptor(s) in non-heart or non-brain vasculature. Binding of the peptide to the vasculature of an organ can be determined by, for example, a binding assay.

The peptides of the present invention can be conjugated to a functional moiety. Due to the selective or preferential homing of the peptides of the present invention to TNF receptor(s) or BDNF receptor(s) in the vasculature of a heart, the functional moiety can be delivered to appropriate areas of the vasculature of a heart. The conjugates can be used to determine the quality (health status) of a heart and/or to deliver therapeutic agents to the appropriate areas of a heart.

In one embodiment, the functional moiety is a detectable marker. The marker can be any marker known to those in the art. For example, the marker can be any marker which emits a detectable signal. Examples of markers include radioisotopes, radionuclides, and fluorophores. An example of a fluorophore include radiofluorinated compounds. Examples of radioisotopes include thallium, iodine¹²⁵, and radioopaque contrast agents.

The markers can be detected by any known method in the art. The method for detecting the marker depends on the marker used. For example, if the markers are radiofluorinated compounds, the markers can be detected by Positron Emission Tomography (PET) scan. If the markers are radioisotopes, the marker can be detected with, for example, a gamma camera. The detectable marker generally allows for detection, visualization, or imaging of the vasculature of a heart.

The homing peptides conjugated to markers can be used to determine the condition of a heart. For example, peptides comprising amino acid sequence QA(Q/E)GQLV (SEQ. ID. NO: 1) bind disproportionately to TNF receptor(s) in the vasculature of a young heart or young portions of a heart vasculature. Disproportionate detection of a marker conjugated to a homing peptide comprising the amino acid sequence QA(Q/E)GQLV (SEQ. ID. NO: 1) indicates a young heart or areas of a heart which are young.

Similarly, peptides comprising amino acid sequence ARRQAV (SEQ. ID. NO: 4) or G(R/W)RFIRV (SEQ. ID. NO: 2) bind disproportionately to BDNF receptor(s) in the vasculature of an old heart or old portions of a heart vasculature. Disproportionate detection of a marker conjugated to a homing peptide comprising the amino acid sequence ARRQAV (SEQ. ID. NO: 4) and/or G(R/W)RFIRV (SEQ. ID. NO: 2) indicates an old heart or areas of the heart which are old.

The terms "young" and "old" as used in this specification, do not refer necessarily to the age of the individual. Instead "young" and "old" refer to the condition of the vasculature of a heart. A young individual, for example, can have a heart which is "old" or have portions of the heart which are "old." In contrast, an old individual can have a heart which is "young" or have portions of the heart which are "young." Young hearts or young portions of a heart vasculature are typically healthy. Old heart or old portions of a heart vasculature are typically unhealthy or damaged.

The disproportionate capacity of a heart or portions of a heart vasculature to bind a peptide comprising the amino acid sequence QA(Q/E)GQLV (SEQ. ID. NO: 1) indicates a young heart or young portions of a heart vasculature. The disproportionate capacity of a heart or portions of a heart vasculature to bind a peptide comprising the

amino acid sequence ARRQAV (SEQ. ID. NO: 4) or G(R/W)RFIRV (SEQ. ID. NO: 2) indicates an old heart or old portions of a heart vasculature.

Administration to a mammal of a peptide comprising the amino acid sequence QA(Q/E)GQLV (SEQ. ID. NO:1) conjugated to a detectable marker can be used to determine whether a heart is young or has young areas. Detection of the marker indicates the capacity of the heart or areas of the heart vasculature to bind TNF α . A disproportionately high binding of the peptide comprising amino acid sequence QA(Q/E)GQLV (SEQ. ID. NO:1) is indicative of a young heart or young areas of a heart vasculature. In contrast, a disproportionately low binding indicates an old heart or old areas of a heart vasculature.

To determine an old heart or old areas of a heart, a peptide comprising the amino acid sequence ARRQAV (SEQ. ID. NO: 4) or G(R/W)RFIRV (SEQ. ID. NO: 2) conjugated to a detectable marker is administered to a mammal. The capacity of the heart or portions of the heart vasculature to bind BDNF can be determined by detecting the marker. A disproportionately high binding of the peptide comprising the amino acid sequence ARRQAV (SEQ. ID. NO: 4) or G(R/W)RFIRV (SEQ. ID. NO: 2) is indicative of an old heart or old areas of a heart vasculature. In contrast, a disproportionately low binding indicates a young heart or young areas of a heart vasculature.

Optimally, to determine the condition of a heart, a peptide comprising the amino acid sequence QA(Q/E)GQLV (SEQ. ID. NO:1) conjugated to a detectable marker and a peptide comprising either the amino acid sequence ARRQAV (SEQ. ID. NO: 4) or G(R/W)RFIRV (SEQ. ID. NO: 2) conjugated to another detectable marker are administered to a mammal. A disproportionately high ratio of binding of the peptide comprising the amino acid sequence QA(Q/E)GQLV (SEQ. ID. NO:1) to binding of the peptide comprising either the amino acid sequence ARRQAV (SEQ. ID. NO: 4) or G(R/W)RFIRV (SEQ. ID. NO: 2) indicates a young heart or young areas of a heart vasculature. A disproportionately high ratio is greater than two, more preferably greater than three, and even more preferably greater than four. Alternatively, a disproportionately low ratio (i.e., ratio less than 2) indicates an old heart or old areas of a heart vasculature.

The order of administration of the peptides is not important in determining the condition of a heart. For example, the selective homing peptide comprising QA(Q/E)GQLV (SEQ. ID. NO: 1) conjugated to a detectable marker can be administered before, after, or simultaneously with the preferential homing peptide comprising the amino acid sequence ARRQAV (SEQ. ID. NO: 4) or G(R/W)RFIRV (SEQ. ID. NO: 2) conjugated to another detectable marker.

Furthermore, the homing peptides conjugated to a detectable marker can also be used to determine the prognosis of cardiac therapy. For example, an image obtained of a heart vasculature before therapy begins, which is indicative of its condition, can be compared to an image obtained after therapy begins. A decrease in the old areas of a heart vasculature or an increase in the young areas indicates an effective therapy.

In another embodiment, the functional moiety is a therapeutic agent. The therapeutic agent can be a small molecule or a biological molecule. A biological molecule is any molecule which contains a nucleic acid or amino acid sequence and has a molecular weight greater than 450.

Biological molecules which can be used as therapeutic agents include viral gene therapy vectors; viruses; nucleic acid molecules and oligonucleotides including antisense and dominant negative molecules; polypeptides, peptides, and proteins. Examples of proteins include microvasculature growth factors such as platelet derived growth factor, vascular endothelial growth factor, angiopoietin, and estrogen.

Small molecules include organic compounds, organometallic compounds, salts of organic and organometallic compounds, saccharides, amino acids, and nucleotides. Small molecules can further include molecules that would otherwise be considered biological molecules, except their molecular weight is not greater than 450. Thus, small molecules may be lipids, oligosaccharides, oligopeptides, and oligonucleotides, and their derivatives, having a molecular weight of 450 or less.

It is emphasized that small molecules can have any molecular weight. They are merely called small molecules because they typically have molecular weights less than 450. Small molecules include compounds that are found in nature as well as synthetic compounds.

In another embodiment, the invention provides methods for delivering a functional moiety to a heart which contain young areas or hearts. Functional moieties, such as detectable markers, can be delivered as described above. The function moiety can also be a therapeutic agent. The therapeutic agent can be used to treat young hearts or young areas of a heart in a cardiac pathology.

Therapeutic agents are targeted to young areas of a heart by administering an appropriate therapeutic agent conjugated to a peptide containing the amino acid sequence QA(Q/E)GQLV (SEQ. ID. NO: 1). Therefore, the therapeutic agent is selectively delivered to the appropriate areas with reduced adverse effects on other organs or areas of the heart.

For example, young areas or hearts can be treated in order to induce proliferation of the cells in the microvasculature of the heart. The proliferation of the young cells of the microvasculature in the heart can compensate for areas damaged by a cardiac pathology.

Therapeutic agents which are useful to treat young areas of a heart or young hearts are known to those in the art and include the therapeutic agents described herein. Therapeutic agents which induce proliferation of heart microvascular cells include, for example, growth factors, such as platelet derived growth factor, vascular endothelial growth factor, and angiopoietin.

In another embodiment, a functional moiety can be delivered to a heart containing old areas or hearts. Functional moieties, such as detectable markers, can be delivered as described herein. The function moiety can also be a therapeutic agent. The therapeutic agent can be used to treat old hearts or old areas of a heart in a cardiac pathology.

Therapeutic agents are targeted to the old area of a heart by administering an appropriate therapeutic agent conjugated to a peptide containing the amino acid sequence ARRQAV (SEQ. ID. NO: 4) or G(R/W)RFIRV (SEQ. ID. NO: 2). Therefore, the therapeutic agent is preferentially delivered to areas damaged by a cardiac pathology.

Therapeutic agents which are useful to treat old areas of a heart in a particular cardiac disease are known to those in the art. The therapeutic agents include, for example, agents that inhibit apoptosis in old hearts or old areas of hearts. Such agents include, for example, estrogen.

Any cardiac pathology that results in damage to a heart can be treated in accordance with the present invention. Cardiac pathologies include, for example, myocardial infarction, myocardial hypertrophy, congenital heart disease, ischemic heart disease, and heart failure.

The conjugates are administered to a mammal in an effective amount. An "effective amount" is the amount of the conjugate that produces a desired effect. An effective amount will depend, for example, on the functional moiety conjugated to the homing peptide, the pathology being treated, and the age, size, and condition of the mammal. An effective amount of a particular conjugate for a particular cardiac pathology is known to, or can routinely be determined by, those skilled in the art.

The conjugate can be administered to a mammal by various routes, including, for example, orally, systemically, or parenterally, such as intravenously. The conjugate can be administered by injection or by intubation.

A mammal can be any mammal. Examples of mammals include primates, such as humans, laboratory animals, such as rats and mice, pet animals, such as dogs and cats, and farm animals, such as cows and sheep.

A conjugate is generally delivered as a pharmaceutical composition. The pharmaceutical composition can, for example, contain a pharmaceutically acceptable carrier. Such carriers are well known in the art and include, for example, aqueous solutions such as physiologically buffered saline or other solvents or vehicles such as glycols, glycerol, and oils such as olive oil.

A pharmaceutically acceptable carrier can contain physiologically acceptable compounds that act, for example, to stabilize or to increase the absorption of the complex. Such physiologically acceptable compounds include, for example, carbohydrates, such as glucose, sucrose or dextrans, antioxidants, such as ascorbic acid or glutathione, chelating agents, low molecular weight proteins or other

stabilizers or excipients. One skilled in the art knows that the choice of a pharmaceutically acceptable carrier, including a physiologically acceptable compound, depends, for example, on the route of administration of the composition.

In another embodiment, mimics of peptides comprising amino acid sequences QA(Q/E)GQLV (SEQ. ID. NO: 1), ARRQAV (SEQ. ID. NO: 4), or G(R/W)RFIRV (SEQ. ID. NO: 2) are discovered. A mimic is any compound having the same three-dimensional structure and binding property as peptides having amino acid sequence QA(Q/E)GQLV (SEQ. ID. NO: 1), ARRQAV (SEQ. ID. NO: 4), or G(R/W)RFIRV (SEQ. ID. NO: 2).

The three-dimensional structure of a peptide can be determined by any known method in the art. Examples of methods for determining the three-dimensional structure of a peptide include, x-ray crystallography and computational methods, such as 3-D structure prediction programs based on, for example, homology modeling. Examples of such programs include SWISS-MODEL, which is available at <http://www.expasy.ch/swissmod/SWISS-MODEL.html>, and CPHmodels, which is available at <http://www.cbs.dtu.dk/services/CPHmodels/>.

Compounds comprising the three-dimensional structure of the homing peptides of the present invention can be identified by any known method in the art. Compounds can be identified by, for example, binding assays, competition assays, spectrophotometry, and x-ray crystallography. The compound can be a small molecule or a biological molecule. The selective and preferential homing capacity of the compounds can be determined as described above. Those compounds which selectively or preferentially home to TNF receptor(s) or BDNF receptor(s) in the vasculature of the heart are mimics.

The peptides of the invention and their mimics can act as an agonist or an antagonist depending on the ability or inability of the peptide or the mimic to activate the receptor. An agonist generally binds to a receptor and stimulates the function of the receptor. A peptide or mimic that activates TNF receptor(s) or BDNF receptor(s) is an agonist. Methods to determine whether a peptide or mimic activates TNF receptor(s) or BDNF receptor(s) are known to those skilled in the art.

An antagonist generally binds to a receptor and inhibits activation of the receptor. A peptide or mimic that inhibits the activation of TNF receptor(s) or BDNF receptor(s) is an antagonist. Method to determine whether a peptide or mimic inhibits the activation of TNF receptor(s) or BDNF receptor(s) are known to those skilled in the art.

An agonist can be useful, for example, in treating myocardial infarction of a young heart or young areas of a heart. Activation of TNF receptor(s) by the agonist can reduce the extent of myocardial damage to the heart.

In contrast, an antagonist can be used to inhibit the activation of TNF receptor(s), for example, to inhibit apoptosis of old hearts or old areas of a heart.

Similarly, an antagonist can be used to inhibit the activation of BDNF receptor(s) in old hearts or old areas of a heart, thereby preventing, for example, infiltration of inflammatory cells and aneurysm formation.

EXAMPLES

Example 1: ψ Y12 with Homology to TNF α binds to Hearts in 3-month Old Mice.

Cardiac-homing phage clone ψ Y12 containing the amino acid sequence QAQGQLV (Figures 3A and 3B), which is homologous to TNF α , identified an aging-associated change in TNF α receptor pathways in the microvasculature of the older heart. *In vivo* injection of the ψ Y12 clone phage confirmed the diminished binding capacity of the TNF α -like phage in the older cardiac microvasculature (Figures 3C-3E), with only minimal binding in the subepicardium of aging hearts. Therefore, TNF receptor is altered in the microvasculature of the older heart.

Example 2: TNF Receptor Expression in Microvasculature of Young and Old Mice.

Immunostaining of 3- and 18-month old hearts revealed TNF receptor 2 in the microvasculature throughout the young and older hearts (Figure 4A and 4B). TNF receptor 1 was present in the microvasculature throughout the young hearts, however, in the older hearts, the receptor was restricted to the subepicardial microvasculature, consistent with the patterning of ψ Y12 in Example 1.

Example 3: Functional Significance of the Change in TNF Receptor in Aging Cardiac Microvasculature.

The functional significance of the changes in TNF receptor in the aging cardiac microvasculature was examined *in vitro*. Cardiac microvascular endothelial cells (CMECs) of 4 and 24-month old F344 rats were isolated and cultured. Briefly, the hearts were removed and minced in endothelial cell medium (DMEM containing 20% FBS, 1% endothelial cell growth factor, 1% endothelial cell growth supplement, 1% BME, 10⁻⁴% heparin and 1% penicillin [10,000 I.U./ml]/streptomycin [10,000 μ g/ml]), and digested with medium containing 0.2% collagenase and 0.005% Dnase, and 5% FBS for 45 minutes with subsequent endothelial cells isolation by PECAM-

mediated magnetic particle collection (Dynabeads). The resultant CMECs (Dil-LDL uptake >95%) were cultured for 2 passages and seeded into 12-well culture dish (10^5 cells/well) and grown to confluence. Medium was changed to serum free-DMEM for 1 hr and then to medium containing 2% FBS \pm TNF α (30 ng/ml) for 3 hr.

In vitro, TNF α induced platelet derived growth factor (PDGF)-B expression in cardiac microvascular endothelial cells (CMECs) from the young, but not in older hearts (Figure 4C).

Example 4: *In Vivo* Response to TNF α

To examine the age-dependent effects of TNF α PDGF-B expression and protection from myocardial necrosis, sets of 4- and 24-month old F344 rats received intramyocardial injections of growth factor. Briefly, the rats were anesthetized and underwent left intercostals thoracotomy. After identifying the left anterior descending artery (LAD), 100 ng of TNF α in 50 μ l PBS or PBS alone was injected through a 30 G needle using a 250 μ l Hamilton syringe. Two injections (25 μ l/injection 2mm apart) were made at mid ventricular anterior wall. The chest wall was then closed, the lungs inflated, the rat extubated, and the tracheotomy closed.

In vivo, TNF α specifically induced increase of PDGF-B in the young hearts, while having minimal effect on the aging tissue (Figures 5A and 5B). Moreover, TNF α markedly reduced the extent of myocardial infarction in the young hearts (Figures 5C and 5D). In the older rats TNF α was harmful, inducing extensive apoptosis in the anterior left ventricular wall and resulting in an 80% mortality within 72 h of coronary occlusion.

Example 5: ψ O40 with Homology to BDNF binds to Heart in Older Mice.

Cardiac-homing phage clone ψ O40 containing the amino acid sequence ARRGGQAV, which is homologous to BDNF (Figures 6A and 6B), identified an aging-associated change in BDNF receptor pathways in the microvasculature of the

older heart. *In vivo* injection of the ψ O40 clone phage combining with immunostaining and recovered ψ O40 phage CFU assay confirmed the more binding capacity of the BDNF-homologous phage in the older cardiac microvasculature while only minimal binding of the subepicardium of the young hearts, suggesting that BDNF receptor(s) may be altered in the microvasculature of the older hearts (Figures 6C and 6D).

Indeed, immunostaining of the BDNF receptor, trkB, demonstrated that the density of the truncated trkB^{trk} receptor was significantly greater in the hearts of older mice and rats compared with the hearts of younger mice and rats, while the distribution of the full-length receptor was similar in both age groups (Figure 7A-D).

Example 6: Functional Significance of the Change in BDNF Receptor in Aging Cardiac Microvasculature.

To probe the age-dependent effects of BDNF on the aging heart, sets of 4- and 24-month old F344 rats (n=3, each set) received intramyocardial injections of the growth factor as described in Edelberg et al. (*Circul.* 2002, 105:608-613). Briefly, the rats were anesthetized and underwent left intercostals thoracotomy. After the left anterior descending artery (LAD) was identified, 1 μ g of rhBDNF (R&D Systems, 248BS005) in 50 μ l of PBS or PBS alone was injected through a 30 G needle using a 250 μ l Hamilton syringe. Two injections (25 μ l/injection 2 mm apart) were made at mid-left ventricular anterior wall. The chest wall was then closed, the lungs inflated, the rat extubated, and the tracheotomy closed.

Rats receiving pretreatment alone (BDNF or control) were sacrificed 24 hr postinjection. The hearts were excised, fixed, sectioned, immunostained for ED1 and ED2, and visualized with DAB. Staining was quantified in the anterior and posterior left ventricular wall at the level of the mid-papillary muscles from each heart, as described in Edelberg et al. (*Circul.* 2002, 105:608-613) [ten high power fields (40X per heart)]. Two investigators performed quantification independently in a blinded fashion.

The functional significance of the alteration in BDNF receptor pathway in the aging cardiac microvasculature was examined *in vivo*. Intramyocardial injection of BDNF 24 hr prior to sacrifice resulted in a similar infiltration of macrophage in both young and older rat hearts, as evidenced by histology and immunostaining for ED2, Figure 8. However, in the older heart BDNF-mediated induction of activated/inflammatory macrophage was significantly greater compared with young hearts, as evidenced by immunostaining for ED1.

Example 7: *In Vivo* Response to BDNF in Young and Old Myocardial Infarction Hearts

The potential effects of BDNF pretreatments were studied in a myocardial infarction model. One day after intramyocardial injection of BDNF (4-month old, n=5; 24-month old, n=5) or control (4-month old, n=5; 24-month old, n=5), the rats were anesthetized, the heart exposed, and the LAD ligated just below (4-month old) or 2 mm below (24-month old, to produce a similar size myocardial infarction to the younger rats) the left atrial appendage with 8-0 nylon sutures. Pallor and regional wall motion abnormality of the left ventricle confirmed occlusion. The chest wall was closed and, after recovery, the rats were returned to the animal facility for 14 d. At the termination of the experiment, the rats were sacrificed and the hearts explanted. The extent of myocardial infarction measured at the level of the mid-papillary heart muscles was scored by Masson's trichrome staining, and the images were analyzed in a blinded fashion employing ImageJ 1.22 software (NIH Image). Infarction size was expressed as a percentage of the total left ventricle myocardial area. Differences were tested for statistical significance by the Wilcoxon rank-sum test. *P* value < 0.05 was considered significant.

In myocardial infarction studies, BDNF demonstrated a similar age-associated effect on cardiac physiology. In the young heart, intramyocardial injection of BDNF 24 hr before coronary occlusion did not alter myocardial infarction size compared with PBS controls (Figure 9). In the older rats, BDNF was deleterious, with significantly more extensive myocardial infarctions compared with hearts injected with control. Moreover, BDNF injection resulted in the formation of ventricular wall

aneurysms (3/5 rats) with persistent macrophage infiltrates two weeks after coronary occlusion (Figure 10).

What is Claimed is:

1. An isolated peptide comprising the amino acid sequence QA(Q/E)GQLV or functional equivalents thereof, wherein said peptide selectively homes to TNF receptor(s) of the vasculature of a heart.
2. A peptide according to claim 1, wherein the amino acid sequence is QAQGQLV.
3. A peptide according to claim 1, wherein the amino acid sequence is QAEGQLV.
4. A peptide according to claim 1, wherein the peptide consists of the amino acid sequence QAEGQLV.
5. A peptide according to claim 1, wherein the peptide consists of the amino acid sequence QAQGQLV.
6. An isolated peptide according to claim 1, wherein said vasculature is microvasculature.
7. An isolated peptide according to claim 1, wherein said peptide further comprises additional amino acid residues.
8. An isolated peptide according to claim 1, wherein said peptide comprises a maximum of approximately 200 amino acid residues.
9. An isolated peptide according to claim 1, wherein said peptide comprises a maximum of approximately 175 amino acid residues.
10. An isolated peptide according to claim 1, wherein said peptide comprises a maximum of approximately 150 amino acid residues.

11. An isolated peptide according to claim 7, wherein said additional amino acid residues constitute a sequence found in human TNF α .
12. An isolated peptide according to claim 1, wherein said peptide further comprises the amino acid residues found approximately at positions 10-15 of human TNF α .
13. An isolated peptide according to claim 1, wherein said peptide further comprises the amino acid residues found approximately at positions 5-15 of human TNF α .
14. An isolated peptide according to claim 1, wherein said peptide further comprises the amino acid residues found approximately at positions 2-15 of human TNF α .
15. An isolated peptide according to claim 1, wherein said peptide further comprises the amino acid residues found approximately at positions 23-60 of human TNF α .
16. An isolated peptide according to claim 1, wherein said peptide further comprises the amino acid residues found approximately at positions 23-120 of human TNF α .
17. An isolated peptide according to claim 1, wherein said peptide further comprises the amino acid residues found approximately at positions 23-150 of human TNF α .
18. An isolated peptide comprising the amino acid sequence ARRQAV or functionally equivalent thereof, wherein said peptide preferentially homes to BDNF receptor(s) of the vasculature of a heart.
19. A peptide according to claim 18, wherein the peptide consists of the amino acid sequence ARRQAV.

20. An isolated peptide according to claim 18, wherein said vasculature is microvasculature.
21. An isolated peptide according to claim 18, wherein said peptide further comprises additional amino acid residues.
22. An isolated peptide according to claim 18, wherein said peptide comprises a maximum of about 200 amino acid residues.
23. An isolated peptide according to claim 18, wherein said peptide comprises a maximum of about 175 amino acid residues.
24. An isolated peptide according to claim 18, wherein said peptide comprises a maximum of about 150 amino acid residues.
25. An isolated peptide according to claim 21, wherein said additional amino acid residues constitute a sequence found in human BDNF.
26. An isolated peptide according to claim 18, wherein said peptide further comprises the amino acid residues found approximately at position 1-4 of human BDNF.
27. An isolated peptide according to claim 18, wherein said peptide further comprises the amino acid residues found approximately at positions 3-4 of human BDNF.
28. An isolated peptide according to claim 18, wherein said peptide further comprises the amino acid residues found approximately at positions 12-30 of human BDNF.
29. An isolated peptide according to claim 18, wherein said peptide further comprises the amino acid residues found approximately at position 12-50 of human BDNF.

30. An isolated peptide according to claim 18, wherein said peptide further comprises the amino acid residues found approximately at position 12-70 of human BDNF.
31. An isolated peptide according to claim 18, wherein said peptide further comprises the amino acid residues found approximately at position 12-90 of human BDNF.
32. An isolated peptide according to claim 18, wherein said peptide further comprises the amino acid residues found approximately at position 12-110 of human BDNF.
33. An isolated peptide comprising the amino acid sequence G(R/W)RFIRV or functional equivalent thereof, wherein said peptide preferentially homes to BDNF receptor(s) of the vasculature of a heart.
34. A peptide according to claim 33, wherein the amino acid sequence is GWRFIRV.
35. A peptide according to claim 33, wherein the amino acid sequence is GRRFIRV.
36. A peptide according to claim 33, wherein the peptide consists of the amino acid sequence GWRFIRV.
37. A peptide according to claim 33, wherein the peptide consists of the amino acid sequence GRRFIRV.
38. An isolated peptide according to claim 33, wherein said vasculature is microvasculature.
39. An isolated peptide according to claim 33, wherein said peptide further comprises additional amino acid residues.

40. An isolated peptide according to claim 33, wherein said peptide further comprises a maximum of about 200 amino acid residues.
41. An isolated peptide according to claim 33, wherein said peptide further comprises a maximum of about 175 amino acid residues.
42. An isolated peptide according to claim 33, wherein said peptide further comprises a maximum of about 150 amino acid residues.
43. An isolated peptide according to claim 39, wherein said additional amino acid residues constitute a sequence found in human BDNF.
44. An isolated peptide according to claim 33, wherein said peptide further comprises the amino acid residues found approximately at positions 80-98 of human BDNF.
45. An isolated peptide according to claim 33, wherein said peptide further comprises the amino acid residues found approximately at positions 60-98 of human BDNF.
46. An isolated peptide according to claim 33, wherein said peptide further comprises the amino acid residue found approximately at positions 40-98 of human BDNF.
47. An isolated peptide according to claim 33, wherein said peptide further comprises the amino acid residues found approximately at positions 20-98 of human BDNF.
48. An isolated peptide according to claim 33, wherein said peptide further comprises the amino acid residues found approximately at positions 4-98 of human BDNF.

49. An isolated peptide according to claim 33, wherein said peptide further comprises the amino acid residues found approximately at positions 106-109 of human BDNF.
50. An isolated peptide according to claim 33, wherein said peptide further comprises the amino acid residues found approximately at positions 106-112 of human BDNF.
51. An isolated peptide according to claim 33, wherein said peptide further comprises the amino acid residues found approximately at positions 106-115 of human BDNF.
52. A conjugate comprising a peptide according to claim 1 and a functional moiety, wherein said peptide selectively homes to TNF receptor(s) in the vasculature of a heart.
53. A conjugate according to claim 52, wherein the amino acid sequence is QAQGQLV.
54. A conjugate according to claim 52, wherein the amino acid sequence is QAEGQLV.
55. A conjugate according to claim 52, wherein the peptide consists of the amino acid sequence QAEGQLV.
56. A conjugate according to claim 52, wherein the peptide consists of the amino acid sequence QAQGQLV.
57. A conjugate according to claim 52, wherein said vasculature is microvasculature.
58. A conjugate according to claim 52, wherein said moiety is a marker.
59. A conjugate according to claim 58, wherein said marker is fluorescent.

60. A conjugate according to claim 58, wherein said marker is radioactive.
61. A conjugate according to claim 58, wherein said moiety is a therapeutic agent.
62. A conjugate according to claim 61, wherein said therapeutic agent is a growth factor.
63. A conjugate according to claim 62, wherein said growth factor is platelet derived growth factor.
64. A conjugate according to claim 62, wherein said growth factor is vascular endothelial growth factor.
65. A conjugate according to claim 62, wherein said growth factor is angiopoietin.
66. A conjugate comprising a peptide according to claim 18 and a functional moiety, wherein said peptide preferentially homes to BDNF receptor(s) of the vasculature of a heart.
67. A conjugate according to claim 66, wherein the peptide consists of the amino acid sequence ARRQAV.
68. A conjugate according to claim 66, wherein said vasculature is microvasculature.
69. A conjugate according to claim 68, wherein said microvasculature comprises microvasculature endothelial cells.
70. A conjugate according to claim 66, wherein said moiety is a marker.
71. A conjugate according to claim 70, wherein said marker is fluorescent.
72. A conjugate according to claim 70, wherein said marker is radioactive.

73. A conjugate according to claim 66, wherein said moiety is a therapeutic agent.
74. A conjugate according to claim 73, wherein said therapeutic agent is estrogen.
75. A conjugate comprising a peptide according to claim 33 and a functional moiety, wherein said peptide preferentially homes to BDNF receptor(s) of the vasculature of a heart.
76. A conjugate according to claim 75, wherein the amino acid sequence is GWRFIRV.
77. A conjugate according to claim 75, wherein the amino acid sequence is GRRFIRV.
78. A conjugate according to claim 75, wherein the peptide consists of the amino acid sequence GWRFIRV.
79. A conjugate according to claim 75, wherein the peptide consists of the amino acid sequence GRRFIRV.
80. A conjugate according to claim 75, wherein said vasculature is microvasculature.
81. A conjugate according to claim 75, wherein said moiety is a marker.
82. A conjugate according to claim 81, wherein said marker is fluorescent.
83. A conjugate according to claim 81, wherein said marker is radioactive.
84. A conjugate according to claim 75, wherein said moiety is a therapeutic agent.
85. A conjugate according to claim 84, wherein said therapeutic agent is estrogen.

86. A method for determining a young heart or young areas of a heart vasculature in a mammal comprising:
- a) administering a peptide comprising the amino acid sequence QA(Q/E)GQLV or functionally equivalent modifications thereof, conjugated to a detectable marker, wherein said first peptide selectively homes to TNF receptor(s) in a vasculature of the heart; and
 - b) detecting the marker;
- wherein a disproportionately high binding of QA(Q/E)GQLV is a young heart or young areas of a heart vasculature.
87. A method according to claim 86, wherein the amino acid sequence is QAQGQLV.
88. A method according to claim 86, wherein the amino acid sequence is QAEGQLV.
89. A method according to claim 86, wherein the peptide consists of the amino acid sequence QAEGQLV.
90. A method according to claim 86, wherein the peptide consists of the amino acid sequence QAQGQLV.
91. A method according to claim 86, wherein said vasculature is microvasculature.
92. A method for determining an old heart or old areas of a heart vasculature in a mammal comprising:
- a) administering a peptide comprising the amino acid sequence ARRQAV or G(R/W)RFIRV or functionally equivalent modifications thereof, conjugated to a detectable marker, wherein said first peptide selectively homes to BDNF receptor(s) in a vasculature of the heart; and
 - b) detecting the marker;
- wherein a disproportionately high binding of ARRQAV or G(R/W)RFIRV is an old heart or old areas of a heart vasculature.

93. A method according to claim 92, wherein the amino acid sequence is ARRQAV.
94. A method according to claim 92, wherein the amino acid sequence is GWRFIRV.
95. A method according to claim 92, wherein the amino acid sequence is GRRFIRV.
96. A method according to claim 92, wherein the peptide consists of the amino acid sequence ARRQAV.
97. A method according to claim 92, wherein the peptide consists of the amino acid sequence GWRFIRV.
98. A method according to claim 92, wherein the peptide consists of the amino acid sequence GRRFIRV.
99. A method according to claim 92, wherein the BDNF receptor is trkB receptor.
100. A method according to claim 99, wherein the trkB receptor is truncated trkB.
101. A method according to claim 92, wherein said vasculature is microvasculature.
102. A method for determining the condition of a vasculature of a heart in a mammal comprising:
 - a) administering a first peptide comprising the amino acid sequence QA(Q/E)GQLV or functionally equivalent modifications thereof, conjugated to a first detectable marker, wherein said first peptide selectively homes to TNF receptor(s) in the vasculature of the heart;
 - b) administering a second peptide comprising the amino acid sequence ARRQAV or G(R/W)RFIRV or functionally equivalent modifications thereof, conjugated to a second detectable marker,

wherein said second peptide homes to BDNF receptor(s) in the vasculature of the heart; and

c) detecting the first and second marker;
wherein a disproportionately high ratio of binding of the first peptide to the second peptide indicates a young heart or young areas of the heart vasculature or wherein a disproportionately low ratio of binding of the first peptide to the second peptide indicates an old heart or old areas of the heart vasculature.

103. A method according to claim 102, wherein the amino acid sequence for the first peptide is QAQGQLV.
104. A method according to claim 102, wherein the amino acid sequence for the first peptide is QAEGQLV.
105. A method according to claim 102, wherein the first peptide consists of the amino acid sequence QAQGQLV.
106. A method according to claim 102, wherein the first peptide consists of the amino acid sequence QAEGQLV.
107. A method according to claim 102, wherein the amino acid sequence for the second peptide is ARRQGAV.
108. A method according to claim 102, wherein the amino acid sequence for the second peptide is GWRFIRV.
109. A method according to claim 102, wherein the amino acid sequence for the second peptide is GRRFIRV.
110. A method according to claim 102, wherein the second peptide consists of the amino acid sequence ARRQGAV.
111. A method according to claim 102, wherein the second peptide consists of the amino acid sequence GWRFIRV.

112. A method according to claim 102, wherein the second peptide consists of the amino acid sequence GRRFIRV.
113. A method according to claim 102, wherein the BDNF receptor is trkB receptor.
114. A method according to claim 113, wherein the trkB receptor is truncated trkB.
115. A method according to claim 102, wherein said vasculature is microvasculature.
116. A method for delivering a functional moiety to a young heart vasculature in a mammal, the method comprising administering a conjugate of claim 48.
117. A method according to claim 116, wherein the amino acid sequence is QAQGQLV.
118. A method according to claim 116, wherein the amino acid sequence is QAEGQLV.
119. A method according to claim 116, wherein the peptide consists of the amino acid sequence QAQGQLV.
120. A method according to claim 116, wherein the peptide consists of the amino acid sequence QAEGQLV.
121. A method according to claim 116, wherein said functional moiety is a therapeutic agent.
122. A method according to claim 121, wherein said therapeutic agent is a growth factor.

123. A method according to claim 122, wherein said growth factor is platelet derived growth factor.
124. A method according to claim 122, wherein said growth factor is vascular endothelial growth factor.
125. A method according to claim 122, wherein said growth factor is angiopoietin.
126. A method according to claim 116, wherein said functional moiety is a detectable marker.
127. A method for delivering a functional moiety to a old heart vasculature in a mammal, the method comprising administering a conjugate of claim 66 or claim 75.
128. A method according to claim 127, wherein the amino acid sequence is ARRQAV.
129. A method according to claim 127, wherein the amino acid sequence is GWRFIRV.
130. A method according to claim 127, wherein the amino acid sequence is GRRFIRV.
131. A method according to claim 127, wherein the peptide consists of the amino acid sequence ARRQAV.
132. A method according to claim 127, wherein the peptide consists of the amino acid sequence GWRFIRV.
133. A method according to claim 127, wherein the peptide consists of the amino acid sequence GRRFIRV.

134. A method according to claim 127, wherein the BDNF receptor is trkB receptor.
135. A method according to claim 134, wherein the trkB receptor is truncated trkB.
136. A method according to claim 127, wherein said functional moiety is a therapeutic agent.
137. A method according to claim 136, wherein said therapeutic agent is estrogen.
138. A method according to claim 127, wherein said functional moiety is a detectable marker.
139. A method for discovering mimics of amino acid sequence QA(Q/E)GQLV or functionally equivalent modifications thereof, comprising:
- a) determining a three-dimensional structure of said sequence;
 - b) identifying compounds comprising said structure; and
 - c) determining the capacity of said compounds for selective homing to TNF receptor(s) in a heart vasculature of a mammal;
- wherein compounds which selectively home to TNF receptor(s) in the vasculature of the heart are mimics.
- 140.. A method according to claim 139, wherein the amino acid sequence is QAQGQLV.
141. A method according to claim 139, wherein the amino acid sequence is QAEGQLV.
142. A method according to claim 139, wherein said vasculature is microvasculature.
143. A method according to claim 142, wherein said microvasculature comprises microvasculature endothelial cells.

144. A method according to claim 139, wherein said mimic is a TNF receptor(s) agonist.
145. A method according to claim 139, wherein said mimic is a TNF receptor(s) antagonist.
146. A method according to claim 139, wherein said amino acid sequence is QAQGQLV.
147. A method for discovering mimics of amino acid sequence ARRQGAV or G(R/W)RFIRV or functionally equivalent modifications thereof, comprising:
- a) determining a three-dimensional structure of said sequence;
 - b) identifying compounds comprising said structure; and
 - c) determining the capacity of said compounds for homing to BDNF receptor(s) in a heart vasculature of a mammal;
- wherein compounds which home to BDNF receptor(s) in the vasculature of the heart are a mimics.
148. A method according to claim 147, wherein the amino acid sequence is ARRQGAV.
149. A method according to claim 147, wherein the amino acid sequence is GWRFIRV.
150. A method according to claim 147, wherein said amino acid sequence is GRRFIRV.
151. A method according to claim 147, wherein the BDNF receptor is trkB receptor.
152. A method according to claim 151, wherein the trkB receptor is truncated trkB.
153. A method according to claim 147, wherein said vasculature is microvasculature.

154. A method according to claim 153, wherein said microvasculature comprises microvasculature endothelial cells.
155. A method according to claim 147, wherein said mimic is a BDNF receptor(s) agonist.
156. A method according to claim 147, wherein said mimic is a or BDNF receptor(s) antagonist.

Figure 1

1 RTPSDKPVAH VVANPQAEQG LQWLNRRANA LLANGVELRD NQLVVPSEGL
51 YLIYSQVLEK GQGCPSTHVL LHTISRIV SYQTKVNLLS AIKSPCQRET
101 PEGAEAKPWY EPIYLGGVFQ LEKGDRLSAE INRPDYLLFA ESGQVYFGII
151 AL

Figure 2

1 HSDPARRGQL SVCDSISEWV TAADKKTAVD MSGGTVTVLE KVPVSKGQLK
51 QYFYETKCNP MGYTKEGCRG IDKRHWNSQC RTTQSYVVRAL TMDSKKRIGW
101 RFIRIDTSCV CTLTIKRGR

FIGURE 3

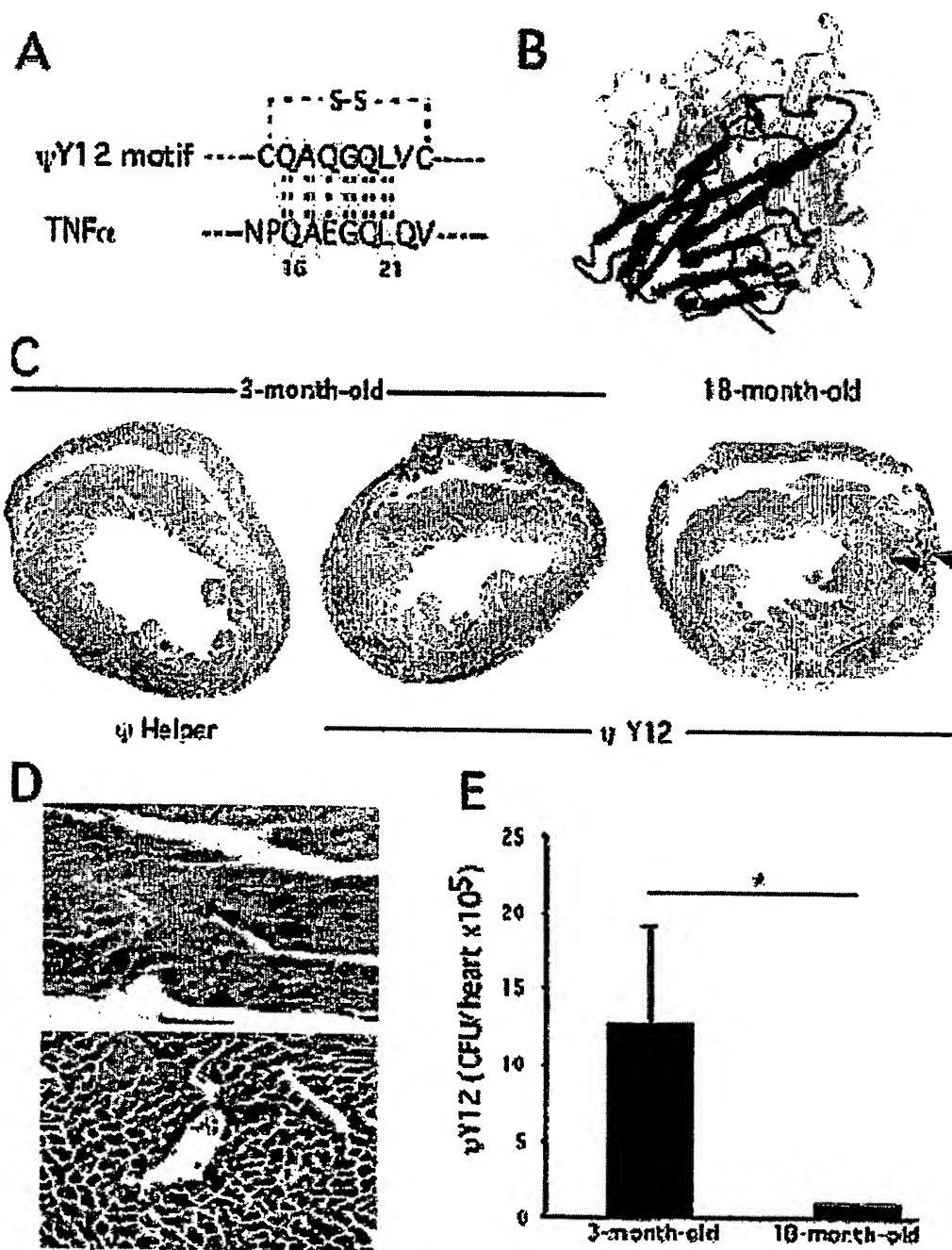


FIGURE 4A

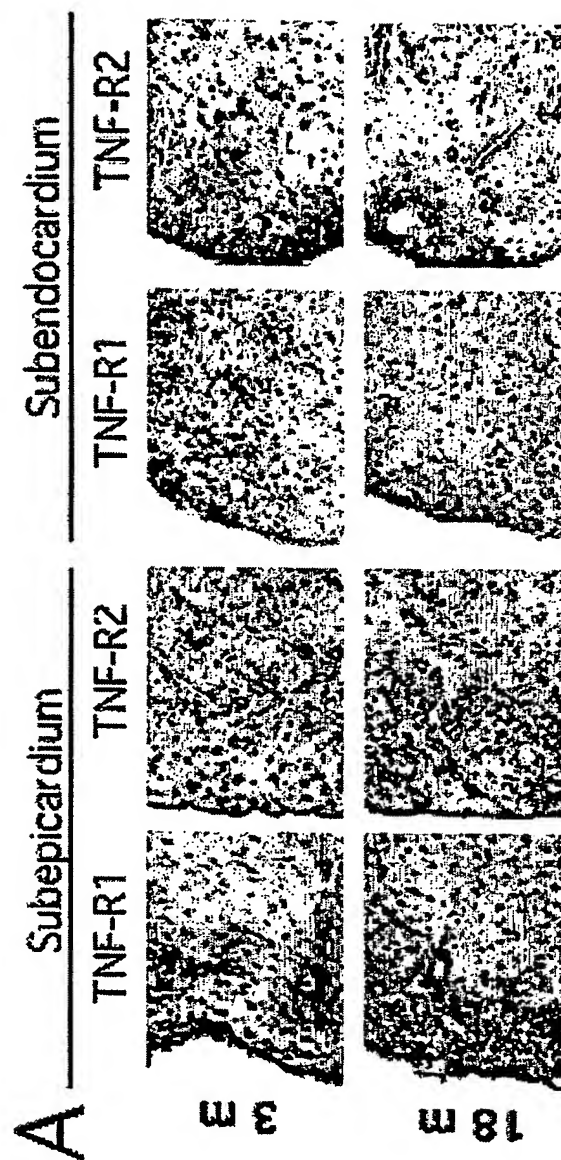


FIGURE 4B and 4C

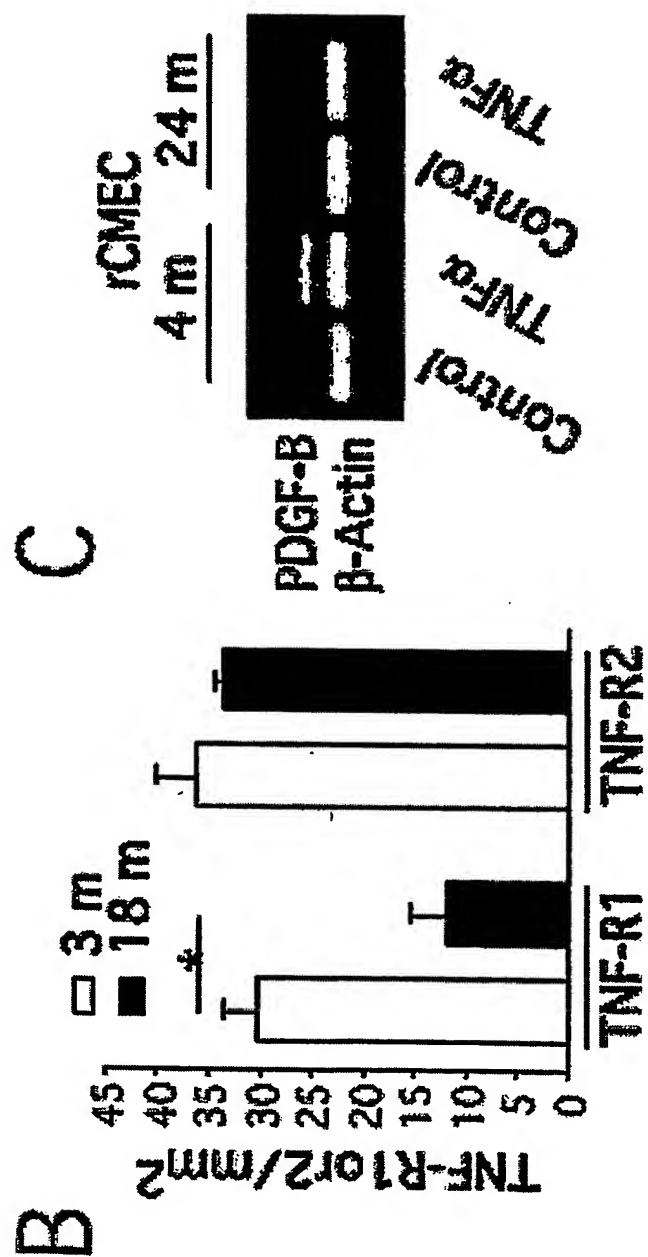


FIGURE 5

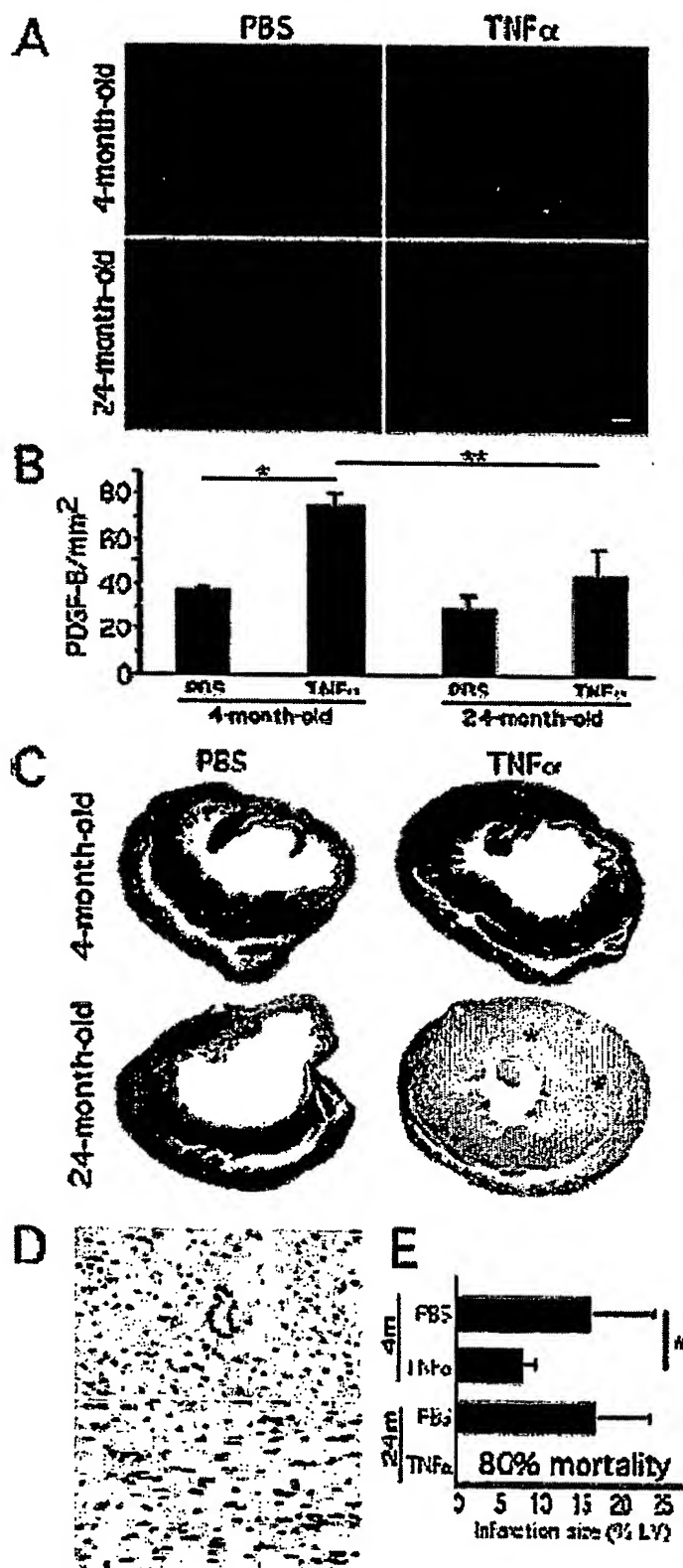


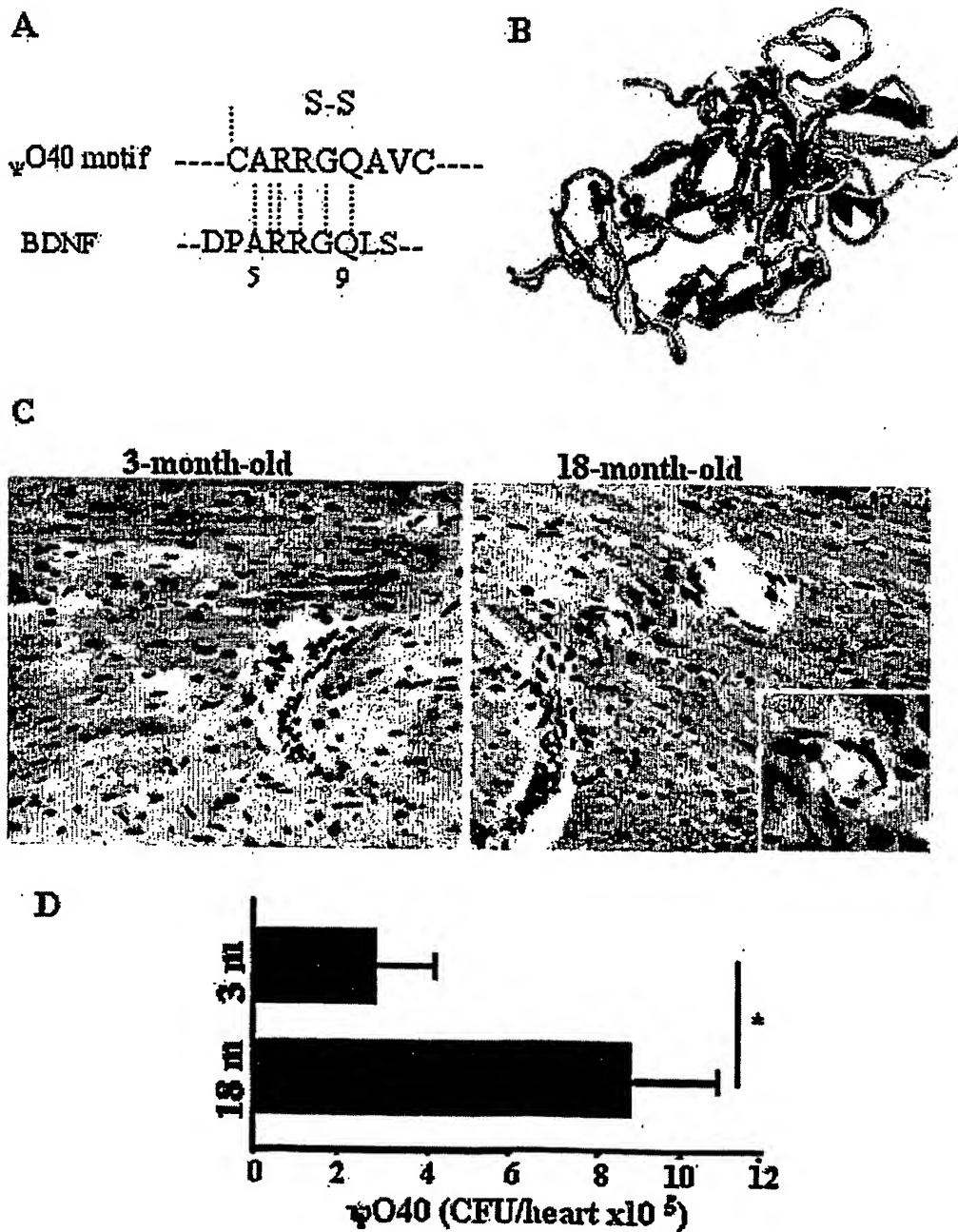
FIGURE 6

FIGURE 7

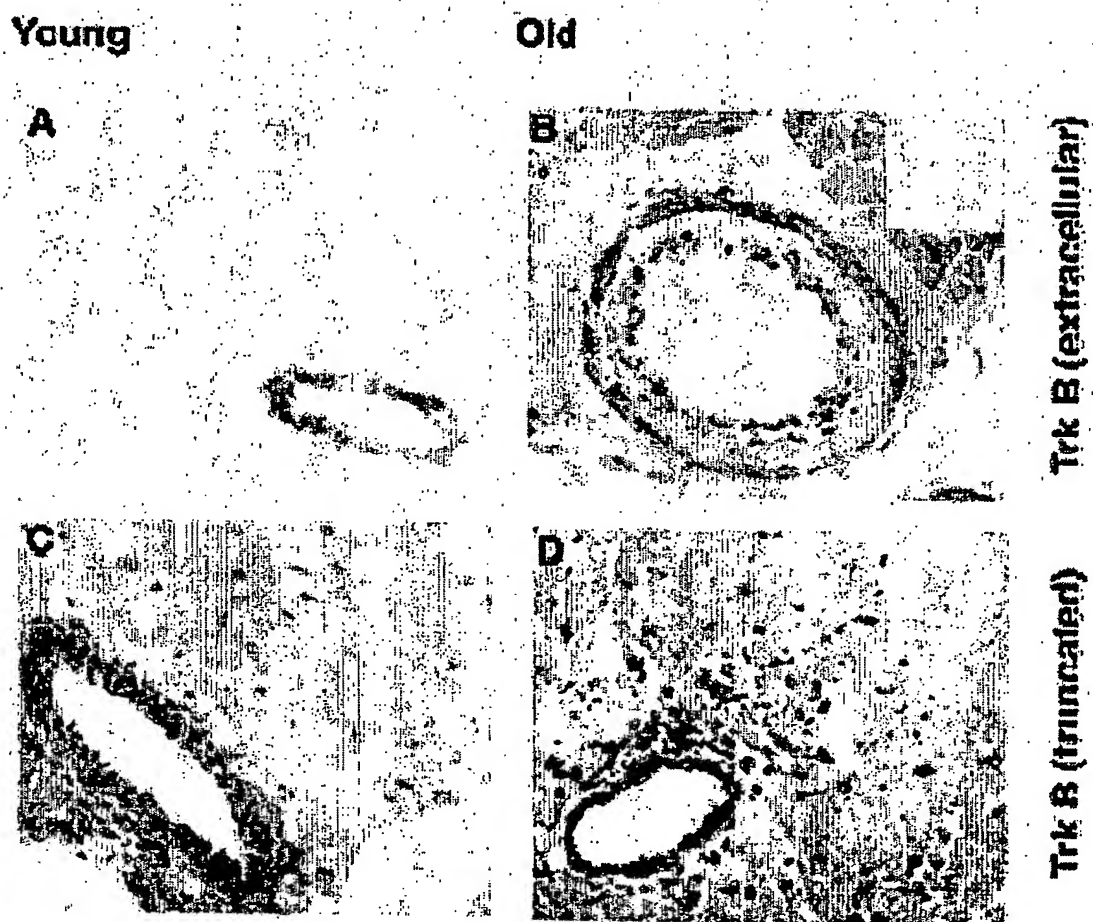


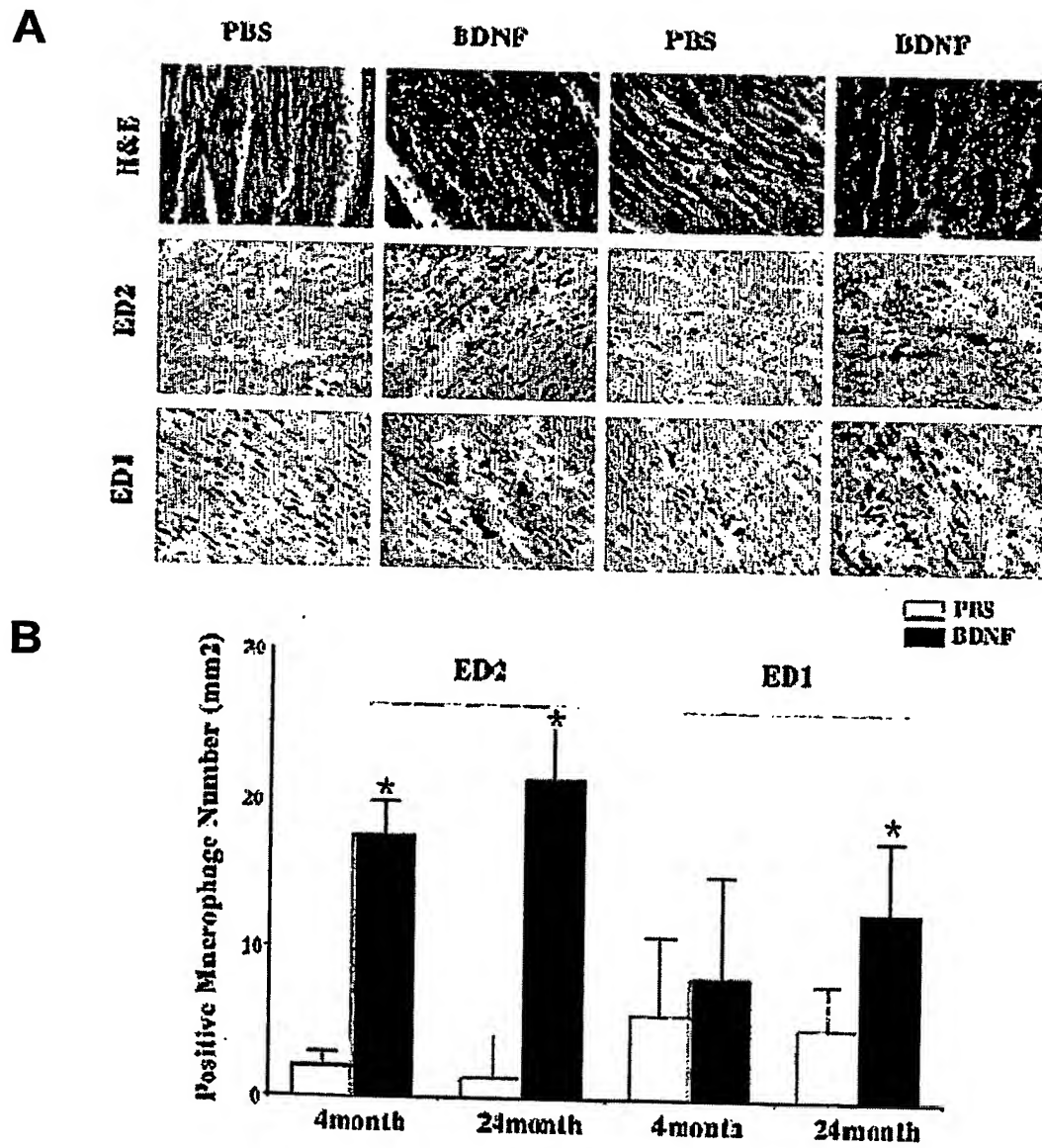
FIGURE 8

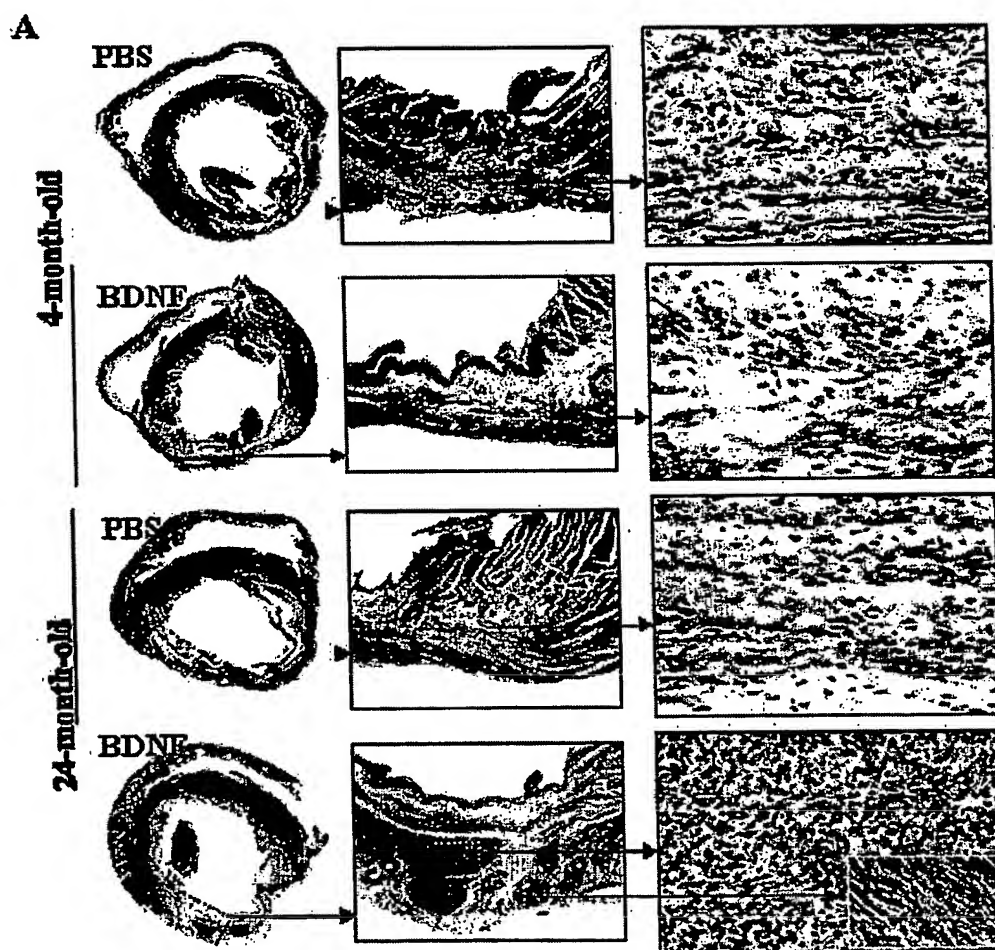
FIGURE 9A

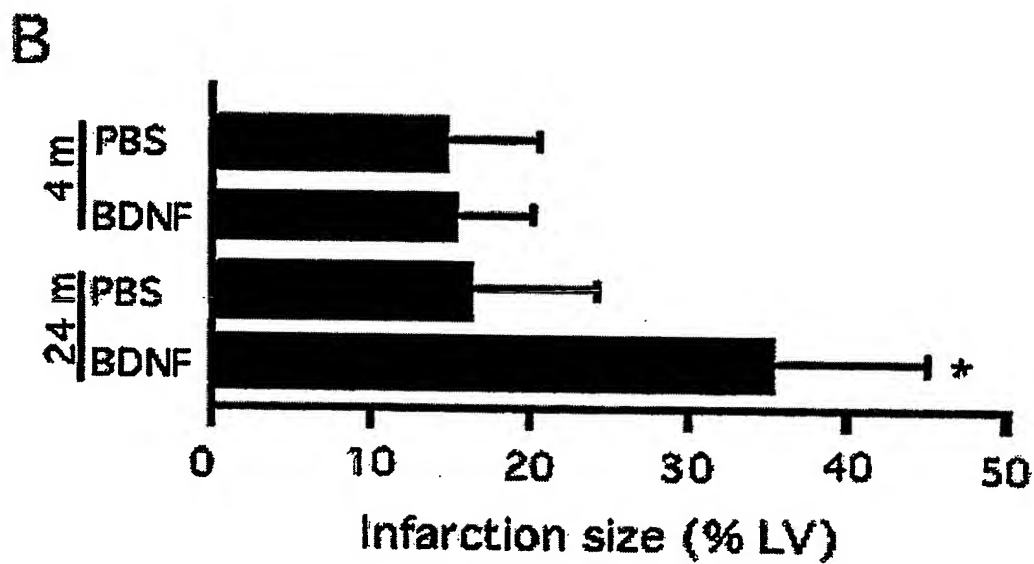
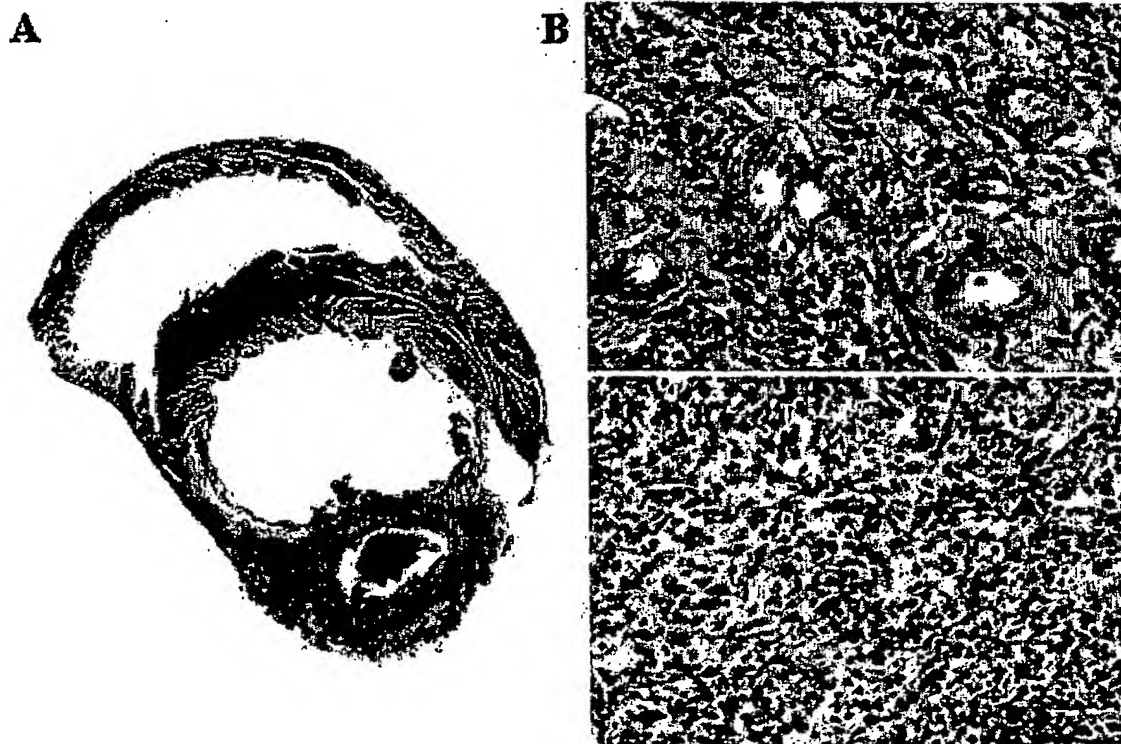
FIGURE 9B

FIGURE 10



INTERNATIONAL SEARCH REPORT

International application No.

PCT/US03/29379

A. CLASSIFICATION OF SUBJECT MATTER

IPC(7) : A61K 38/00

US CL : 530/329

According to International Patent Classification (IPC) or to both national classification and IPC

B. FIELDS SEARCHED

Minimum documentation searched (classification system followed by classification symbols)

U.S. : 530/329

Documentation searched other than minimum documentation to the extent that such documents are included in the fields searched

Electronic data base consulted during the international search (name of data base and, where practicable, search terms used)

Please See Continuation Sheet

C. DOCUMENTS CONSIDERED TO BE RELEVANT

Category *	Citation of document, with indication, where appropriate, of the relevant passages	Relevant to claim No.
Y	US 6,303,573 B1 (RUOSLAHTI et al.) 16 October 2001 (16.10.2001), entire document (especially col. 3, lines 61-67 to col. 4, lines 1-8).	1-156
Y	US 2002/0044944 A1 (NAKAMURA et al.) 18 April 2002 (18.04.02), especially para. 0004 and claims.	1-156

☐ Further documents are listed in the continuation of Box C.

☐ See patent family annex.

* Special categories of cited documents:

"A" document defining the general state of the art which is not considered to be of particular relevance

"E" earlier application or patent published on or after the international filing date

"L" document which may throw doubts on priority claim(s) or which is cited to establish the publication date of another citation or other special reason (as specified)

"O" document referring to an oral disclosure, use, exhibition or other means

"P" document published prior to the international filing date but later than the priority date claimed

"T" later document published after the international filing date or priority date and not in conflict with the application but cited to understand the principle or theory underlying the invention

"X" document of particular relevance; the claimed invention cannot be considered novel or cannot be considered to involve an inventive step when the document is taken alone

"Y" document of particular relevance; the claimed invention cannot be considered to involve an inventive step when the document is combined with one or more other such documents, such combination being obvious to a person skilled in the art

"&" document member of the same patent family

Date of the actual completion of the international search

23 February 2004 (23.02.2004)

Date of mailing of the international search report

11 MAR 2004

Name and mailing address of the ISA/US

Mail Stop PCT, Attn: ISA/US
Commissioner for Patents
P.O. Box 1450
Alexandria, Virginia 22313-1450

Facsimile No. (703) 305-3230

Authorized officer

Dorrie Bell-Harris for
Maury Aude

Telephone No. 703-308-1123

INTERNATIONAL SEARCH REPORT

PCT/US03/29379

Continuation of B. FIELDS SEARCHED Item 3:

EAST (Internal UPSTO); Medline.

[*It is noted that there was no sequence listing found in the claims, although peptide sequences longer than 4 amino acids were claimed. It is suggested that Applicant submit a sequence list/computer readable form corresponding to these].